

Oceanography and Environmental Sciences at Miami

Editor's note: This article on the University of Miami's Marine and Freshwater Biomedical Sciences Center (UM MFBS) is the fifth in a series that appears intermittently in NIEHS News. The series highlights the activities of Environmental Health Sciences and Marine and Freshwater Biomedical Sciences Centers. The first article in the series appeared in volume 101, number 7.

Stationed on the white sands of Virginia Key overlooking Biscayne Bay, the Rosenstiel School of Marine and Atmospheric Sciences is home to the University of Miami's Marine and Freshwater Biomedical Sciences Center. The center is jointly funded by the NIEHS and the University of Miami, and serves as a focal point for marine-related health issues for the temperate United States, Central and South America, and the Caribbean basin. The center plays a leading role in research on natural seafood toxins, and with the advent of seafood safety legislation in early 1995, research at the center is critical in providing a science-based approach to seafood safety.

The Rosenstiel School has been involved in research related to the tropical and subtropical oceans and atmosphere, its flora and fauna, since its creation in 1943; it is the third largest oceanographic institution in the United States. The UM MFBS Center has funded programs to investigate the use of marine species as models of

human diseases, molecular mechanisms and orphan receptors relating to marine toxins, neurophysiology, metabolism, and immunology. The UM MFBS is the

newest of five marine centers and is directed by Daniel G. Baden. Associate directors are Patrick Walsh and David Adams. The center fosters collaboration among eight investigators from four university departments, with ten scientists carrying out pilot projects.

Four center postdoctoral fellows provide additional expertise in selected research areas. The collaborations resulting from center activities extend to all campuses of the University of Miami and to colleagues elsewhere in the United States and abroad.

A Bolt of Lightning

Insight to marine models and toxins is often serendipitous, arising from direct observation of the marine environment. Marine organisms react to their environment in much the same way as do humans, and the negative effects of exposure to environmental agents are graphically displayed. For example, the damselfish neurofibromatosis model was discovered during diving expeditions to observe the territorial and mating behavior of this fish. A pronounced concentration of fish with neurofibromatosis in certain geographic areas led to the hypothesis that an environmental or infective agent was responsible

for the disease. This discovery eventually led to the development of a unique model system for a human cancer.

Red tides and the massive destruction they produce is another example. As a result of studies of fish kills and "mysterious toxic aerosols" (at one time thought to be leaking submerged nerve-gas containers), specific natural biotoxins were identified as the causative agents. Later, the toxins were shown to accumulate in food sources and cause human illness upon consumption. In other cases, the toxic marine food source was known and back-tracking through the marine food web led to the identification of the toxigenic organism.

Thus, the marine environment provides highly sensitive systems for study, both as human disease mimics and as vectors of some of the most toxic materials known to man. In contrast to work carried out with their terrestrial counterparts, marine species offer the opportunity to bathe the specimen in the toxicant of interest and to provide a uniform and reproducible concentration or gradient for acute and chronic studies. Marine species were first exploited as models early in this century (squid giant axons) and have evolved as sentinel species (see "Assessing a Damaged Earth," *EHP* 102:532); their promise as models of human health effects is rediscovered every decade or so (see NIEHS News, *EHP* 102:272). At the UM MFBS, all marine organisms are considered as potentially relevant to human health, whether as a model system or as indicators of pollution and toxin contamination.

Metabolic Poisons

Daniel Baden has been studying marine toxins that accumulate in seafood since the mid-1970s. Baden began his research as an NIH graduate trainee in molecular enzymology and received an NIEHS Young Environmental Scientist award in December 1979. Intoxication arising from the consumption of seafood is now acknowledged worldwide as an underreported health effect, and the U.S. Food and Drug Administration has developed HACCP (Hazard Analysis Critical Control Point) regulations to be implemented in 1995 to address the issues of seafood safety. Central to Baden's work is delineation of the molecular mechanisms by which marine toxins produce their deleterious effects on humans. Marine toxins are responsible for an estimated 62% of all seafood-related illnesses, or 38% of all meat-related intoxication, but they are of low molecular weight and difficult to

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UM MFBS

Lab by the sea. The Rosenstiel School of Marine and Atmospheric Science on Virginia Key houses the University of Miami's Marine and Freshwater Biomedical Sciences Center.

detect. More than 90,000 people are estimated to be afflicted annually by natural marine toxins in food. The numbers are only an approximation; domestic and international epidemiological records are incomplete because physician reporting is not mandatory. Intoxication ranges from gastrointestinal upset in mild cases to respiratory paralysis, dementia, and long-term recurrent symptoms or death in the most severe cases. Health effects resulting from chronic exposure have not yet been addressed adequately.

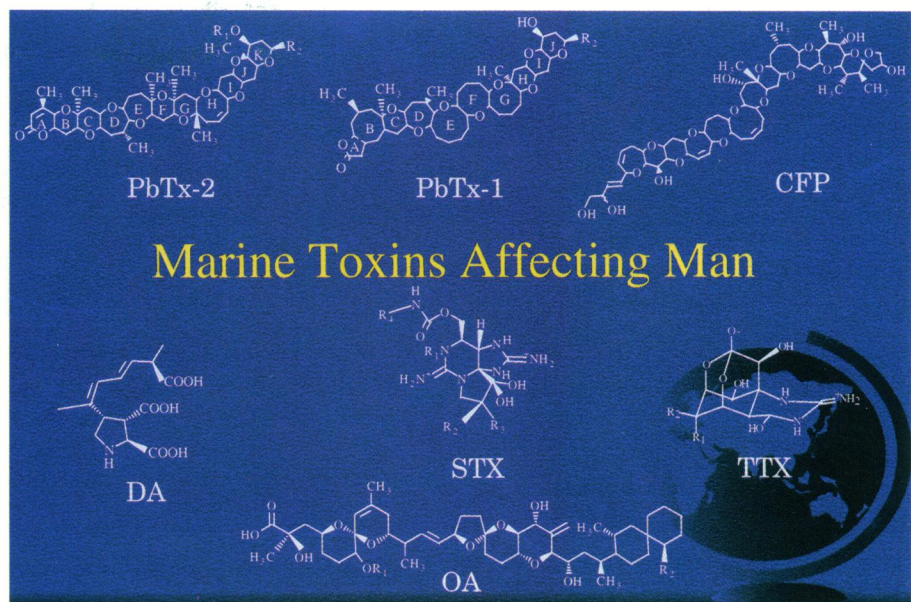
Each toxin binds to a specific class of receptors and initiates the cascade of events which ultimately leads to illness. Four types of receptor interactions account for all known maladies; more than 60 individual toxins and derivatives have been described. The okadaic acid class interacts with protein phosphatases and inhibits normal protein activation/inactivation cascade systems. Domoic acid and its derivatives bind with high affinity to central nervous system glutamate receptors and cause permanent long- and short-term memory loss.

The last two toxin groups bind to specific orphan receptors located on voltage-sensitive sodium channels, which are responsible for nerve signal conduction. The ion channel inhibitors represented by saxitoxin and tetrodotoxin are responsible for paralytic shellfish poisoning and puffer fish poisoning, respectively. Brevetoxins and ciguateras induce neurotoxic shellfish poisoning and ciguatera, respectively, by causing spontaneous and uncontrolled electrical activity in nerves.

Researchers at the Miami center use a unique approach to decipher the molecular mechanism of each toxin type. Chemical modification of each toxin is used to determine the requisite portions of the molecules. Each synthesis is evaluated and guided by molecular computer modeling; binding constants for each toxin-receptor complex, are measured along with evoked and spontaneous electrical activity; and toxicology of the *Gambusia* fish is used as a point of reference.

Organic chemists Robert Gawley, Kathleen Rein, and Thomas Mende, electrophysiologists David Adams and Gerhard Jeglitsch, and biochemists Vera Trainer and William Catterall have provided data to model the interaction of polyether toxins brevetoxin and ciguatoxin with the voltage-sensitive sodium channel. Accurate predictions of toxicity and binding affinity are now possible based on modeled toxins.

The results of this work have immediate applications for development of test kits for public health and clinical use and provide new data essential for clinical evaluation and treatment of afflicted individuals. Physician



Ocean predators. (Clockwise from top left) Neurotoxic shellfish poisons PbTx-2 and PbTx-3; ciguatera toxin, CFP; puffer fish poison, TTX; paralytic shellfish poison, STX; diarrhetic shellfish poison, OA, and amnesiac shellfish poison, DA.

Lora Fleming has used the assays and results of the toxicity studies in the emergency room to treat patients and collect serum samples for biomarker evaluation in cases of ciguatera fish poisoning. A Ciguatera Network, consisting of biomedical scientists, a marine ecologist and fisheries biologist, area physician, an epidemiologist, and public health authorities, was established in 1991. This network provides general information, sample analysis, treatment, and a repository for official reporting.

Pilot projects on epidemiology and neuropsychopharmacology seek to more

fully document and research marine seafood intoxication in the United States and the Caribbean. Pilot project collaborator Ernest Lee and graduate student Masao Kinoshita have provided new insight into how okadaic acid interferes with protein dephosphorylation during diarrhetic shellfish poisoning. Postulating that okadaic acid acts as the regulator of dinoflagellate protein phosphatases, Lee and Kinoshita believed that the toxins substituted as "charlatan" regulators, binding to the same site as the normal regulator in afflicted humans—only more tightly. Computer-



No snail's pace. The *Aplysia californica* mollusk model is rapidly advancing knowledge of memory and learning.



Damsel in distress. A healthy biocolor damselfish faces off against a tumored member of the species used as a model of human cancer.

aided modeling has presented an overall topography of the most potent toxins and has provided evidence that homologous function of the freshwater microcystins, okadaic acid, and the natural inhibitors in mammalian cells may be a result of their nearly identical shapes, and, hence, recognition by protein phosphatases.

In an interesting twist of toxin transformed into research tool, some natural marine toxins are being promoted as molecular "measuring tapes" for complex glycoproteins like ion channels. Using more than 20 specialized toxin derivatives, center scientists Patrick Walsh and Barbara Washburn are researching toxin metabolism, accumulation and depuration, organ and tissue storage, and metabolite detection. Further development of enzyme-aided organic synthesis by postdoctoral fel-

low Kathleen Rein promises many more new derivatives, which can be designed to modulate ion channels, enzymes, and receptors in predictable ways. New developments at the center are being shared with local industries via a Technology Transfer program.

They Multiply Like Rabbits

The Rosenstiel campus is also home to the university's Aplysia Resource Facility, the largest of its kind in the world. The present demand for Aplysia (*Aplysia californica*) sea hares began in the mid-1970s when Eric Kandel's group at Columbia University began to establish the utility of these organisms in neurosciences, particularly as a model for memory and learning. The nervous system of Aplysia is ideal for this work because there are a small number of large, consistently identifiable neurons in the ganglia, which are linked to relatively simple behaviors. Initially working in Kandel's group, the UM facility manager,

Tom Capo, was responsible for much of the basic husbandry research involved in completing the life cycle of the animal in culture; particularly noteworthy were developments in larval metamorphosis and settling. Since the facility moved to UM in late 1988 under sponsorship by Howard Hughes Medical Institute, Capo and his group have consistently produced up to 20,000 animals per year for scientific research. Their success is largely due to the predictable day length and temperatures in Miami, which allow them to produce the 400 lb per week of red seaweed necessary to feed the sea hare.

Models of Human Disease

Fishes of the family Batrachoididae, represented by the toadfishes, have been the subjects of biomedical research for nearly a century. These advanced teleost (bony) fishes have a face only a mother toadfish could love, and may seem to be little more than a nuisance to anglers. To scientists, however, they are interesting model systems for a number of physiological, metabolic, and toxicological studies. As early as 1959, it was known that the oyster toadfish, *Opsanus tau*, a resident of the northeastern seaboard, has a specialized sonic muscle which is among the fastest-contracting muscle in the animal kingdom. Faster even than insect flight muscle, the sonic muscle has a number of unique biochemical aspects which led to the discovery of several unifying concepts in muscle physiology. (In another species of the family, *Porichthys notatus*, the plainfin midshipman, the muscle is also responsible for the disturbing "hum" heard by residents of Northern California marinas.)

Recent research at the UM MFBS Center has focused on a related species, the gulf toadfish, *Opsanus beta*, which inhabits the shallow marine waters of Florida and

HACCP and Seafood Safety

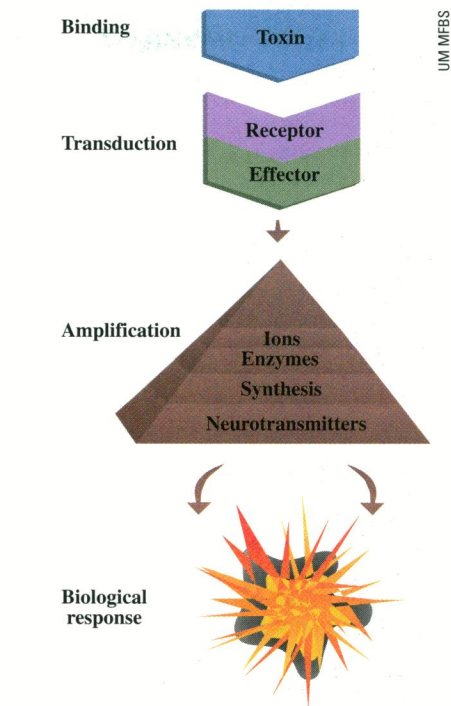
Since 1906, the Food and Drug Administration has been in charge of seafood safety by ensuring the wholesomeness and proper labeling of seafood products. Beginning in 1994, the FDA has implemented an even stricter set of guidelines to ensure safer seafood. These guidelines are called the Hazard Analysis Critical Control Point (HACCP) system. They were originally developed by the Pillsbury Company and NASA in 1959 to ensure the quality of foods taken into space. By identifying critical control points in food processing and addressing evaluation criteria, near 100% quality assurance could be approached. The seafood HACCP system was developed to set the stage for safer seafood by making the industry, namely, seafood processors, distributors, and importers, identify potential points in their operations where a failure could result in health hazards, apply control points to prevent those hazards; and maintain records to ensure that the controls are effective. Among the hazards HACCP addresses are spoilage, microbiological flora such as bacteria and viruses, and chemical hazards such as PCBs and natural marine toxins. When fully implemented, HACCP is expected to reduce the overall incidence of seafood intoxication, while reducing the estimated \$191 million in annual liability exposure to the industry.

the Gulf of Mexico. A decade ago, NIH (*National Cancer Institute Monograph* 65, 1984) recognized that the gulf toadfish and other cold-blooded "poikilothermic" species could be useful models in the study of carcinogenesis and in carcinogenicity testing. Since most fish have body temperatures lower than that of mammals, study of the effects of temperature were called for to aid in extrapolating results with piscine systems to mammalian systems. In a study funded by NIEHS, Walsh and postdoctoral fellow Chris Kennedy found that elevated environmental temperature led to changes in the uptake and metabolism of the carcinogen, benzo[*a*]pyrene (BaP) by the gulf toadfish and shifted metabolic detoxification pathways toward enhanced production of BaP tetrols, breakdown products of highly carcinogenic BaP metabolites. The shift occurred regardless of whether the temperature change was sudden or gradual. These observations may in part explain why fish appear to be less susceptible to chemical carcinogenesis at lower temperatures, and the observations also have implications for the health effects of global warming being currently debated. As a by-product of their research, Walsh and Kennedy developed methods to keep toadfish hepatocytes in culture, with stable cytochrome P450 levels, for up to a month, a feat that has been difficult to achieve with mammalian hepatocytes.

The gulf toadfish has also been used to examine brevetoxin metabolism. Using radioactive brevetoxin, researchers at the UM Center traced the tissue distribution and fate of brevetoxin. They found that, although much of the brevetoxin was metabolized and excreted in bile, a significant proportion of brevetoxin its metabolites remained in the muscle. Thus, brevetoxin and other related marine toxins may have a substantial residence time in seafood. A second practical conclusion from this research was that human clinical studies of the fate of marine toxins could perhaps benefit by including fecal sampling for metabolites excreted via the hepatobiliary route. Identification of the specific metabolites of brevetoxin and the mechanisms by which brevetoxin induces cytochrome P450s are topics of current research.

Another peculiar trait of the gulf toadfish is being exploited in studies of ammonia toxicity. Although most aquatic species excrete ammonia as their principal nitrogenous waste product, the gulf toadfish is unique in that it can excrete waste as urea, just as mammals do. The gulf toadfish should thus be a good model for the study of ammonia neurotoxicity and detoxification pathways.

Postdoctoral fellow Jeffrey Duffy is



Dose and response. Exposure to an environmental toxin can produce a chain of events resulting in adverse effects.

examining the effects of ammonia and other neurotoxins on brain glial fibrillary acidic protein (GFAP) in the gulf toadfish. In mammals, after a neurotoxic event damages neurons, cells proliferate to, in essence, "fill in the space." These cells are rich in GFAP, and thus elevated GFAP levels can be used as an indicator of neurotoxicity. Preliminary observations suggest that the toadfish can withstand levels of ammonia 10-fold greater than those which produce neurotoxicity in mammals and other fish. Further experiments are expected to determine if this resistance to ammonia toxicity is due to enhanced detoxification abilities, unique characteristics of toadfish nerve cells, or other factors. Understanding the factors that confer resistance to ammonia toxicity should lead to a better understanding of brain ammonia toxicity and inborn errors in urea metabolism in mammals. The usefulness of GFAP as an indicator of neurotoxicity in environmentally stressed fish is also being explored and presents opportunities for defining new sentinel species for environmental monitoring.

In the late 1970s, Michael Schmale began to notice a disease affecting one species of fish, the bicolor damselfish (*Pomacentrus partitus*), on Florida reefs. Observations by other scientists suggested that this disease had been present on Florida reefs since the 1960s. In collaboration with George Hensley, a pathologist at

the University of Miami School of Medicine, Schmale determined that the damselfish had a cancerous disease that was similar to a disease in humans, neurofibromatosis. Neurofibromatosis type 1 (NF1; there are several other, less common types) is an inherited disease with an incidence rate of about 1 in 3,000 births. The damselfish disease was named damselfish neurofibromatosis (DNF) because it shared some key features with NF1, such as the type of tumor (plexiform neurofibroma). Neurofibromas arise from the Schwann cells and fibroblasts that ensheath the axons of peripheral nerves. As these tumors develop, affected nerves enlarge in diameter and length due to the cell proliferation. This process can eventually lead to very large, disfiguring, and sometimes life-threatening tumors. Recently, the gene responsible for NF1 was localized on chromosome 17, sequenced, and its protein product, neurofibromin, identified. In spite of this rapid progress at the molecular level, the mechanisms responsible for tumor formation and the reasons for its variable expression in individuals with similar mutations remain unknown. For many years researchers studying NF1 have been frustrated by the lack of a good animal model.

For the last 14 years, Schmale and co-workers have been developing DNF into an animal model for the study of NF1. A wide range of studies have been completed or are in progress documenting the distribution of DNF in natural populations, its histopathology, ultrastructure, immune system effects, and etiologic agents of this disease. These investigations have revealed that DNF is most likely caused by a virus. Although this etiology is quite different from the genetic mutations responsible for NF1 in humans, there are a number of advantages to using models based on infectious agents. The ability to transmit DNF in the laboratory provides a rapid and reliable means of monitoring all stages of its development in different hosts, under different environmental conditions, or with different preparations of the etiologic agent. Five cell lines developed from the tumors have been shown to be composed entirely of neoplastic Schwann cells and to be capable of inducing neurofibromas when injected into healthy fish. This combination of *in vitro* and *in vivo* model systems provides a variety of tools to study the mechanisms by which the agent responsible for DNF transforms these cells. Electrophysiological studies have demonstrated significant differences in the kinetics of potassium currents in the membranes of normal and neurofibroma-derived Schwann cells, suggesting that alterations in potassium channels occur during tumorigenesis. Preliminary observations

have indicated that fish in the wild live for approximately 1 year after developing the first tumors. When these fish are transferred to the laboratory, their rate of tumor development slows considerably. Although tumor remission has never been observed, these diseased fish may live for several years in the laboratory, suggesting that the rate of progression of DNF is affected by environmental factors.

Despite the large number of studies of carcinogenesis in fishes, few have investigated immune system responses at any level. The immunology of DNF is being examined in collaboration with another center investigator, Churchill McKinney of the Department of Microbiology and Immunology of the University of Miami School of Medicine. Immune responses in damselfish are being investigated from three perspectives: 1) the effects of the disease process on the various components of the immune response, 2) anti-tumor activity of leukocytes in these fish, and 3) the type, distribution, and function of tumor-infiltrating leukocytes in DNF. The first group of studies has demonstrated that damselfish in advanced stages of DNF are often severely immunosuppressed relative to healthy individuals. Tumor-bearing, but not normal fish, have cells capable of killing tumor targets. These cytotoxic responses have both nonspecific and specific components, indicating that at least two populations of cytotoxic cells are present. Finally, histological studies have shown that neurofibromas in DNF are characterized by a conspicuous granulocytic infiltrate, as are neurofibromas in NF1. In humans these cells are basophilic mast cells, whereas in damselfish they are eosinophilic granule-containing cells (EGCs). The density of EGCs in tumors depends on the nature of the inoculum used to induce the tumors. Ongoing studies are focusing on the identity and control of the cytotoxic cells evoked in DNF and the role of the EGCs in tumorigenesis.

Subtropical and Tropical Bioindicators

Numerous studies of marine and estuarine animals in temperate, heavily polluted environments have demonstrated that pollution can have severe effects on the health of the resident fauna. Researchers have developed bioindicators of stress for different species in different habitats. The usefulness of these indicators in subtropical or tropical environments, especially those that are not severely polluted, has not been adequately investigated. Biscayne Bay is a shallow estuarine/marine bay adjacent to Miami. The sediments in some areas of the bay are moderately polluted with petroleum hydrocarbons and heavy metals. UM

center scientists have conducted a survey of fish health in Biscayne Bay. The study revealed that up to 11% of fish in four target species (sea bream, blue-striped grunt, pinfish, and grey snapper) exhibit some type of morphological abnormality. The most common types of abnormalities were deformation or loss of spines of the dorsal fin or disorientation of patches of scales. Significant differences were found in the prevalence of these abnormalities between different collection sites in the bay. Ongoing studies are aimed at identifying environmental factors associated with high prevalences of these abnormalities. Correlations between the prevalence of abnormalities and distribution of contaminants in sediments are being investigated further via laboratory exposures. In addition, the usefulness of a number of physiological measures as bioindicators are being evaluated in different species under a variety of environmental conditions in the laboratory and the field. Levels of phase I and II detoxification enzymes, lipid peroxidase, metallothioneins, heavy metal concentrations, phagocytic activity of macrophages, and levels of heat-shock proteins have been monitored.

Timing Is Everything in New Breast Cancer Studies

The timing of exposures to environmental agents during critical developmental periods as it pertains to risk of abnormal development and breast cancer is the focus of recently funded grants from NIEHS. The grants were made in response to applications received through a grants Request for Applications (RFA ES 94-004). The RFA also focused on studies to understand the cellular, genetic, and hormonal effects of environmental agents on the normal growth and development of the mammary gland and to study the role of environmental factors on the development of breast cancer. The grants also intend to further understanding of the mechanism of action of environmental exposures to agents such as organochlorine pesticides, polyaromatic hydrocarbons, and radiation in the development of breast cancer.

The six grantees, their affiliations, and the titles of their proposed studies are:

- Scott Burchiel, University of New Mexico: Mammary Cell Signaling Produced by Environmental Agents;
- Chia-Cheng Chang, Michigan State University: Mechanisms of Environmental Agent-induced Breast Cancer;
- Colin Jefcoate, University of Wisconsin: Organochlorine Compounds and Human Breast Cytochrome P450;
- Coral Lamartiniere, Louisiana State University: Timing of Environmental Chemicals in Breast Cancer;

- Robert Liburdy, Lawrence Berkeley Laboratories: Environmental Magnetic Fields and Human Breast Cancer;
- Jose Russo, Fox Chase Cancer Center: Susceptibility of the Breast to Environmental Carcinogens.

NIEHS, NCI, and NIA Collaborate on Breast Cancer

NIEHS is collaborating with the National Cancer Institute and the National Institute on Aging in funding four developmental center grants at NCI-designated Cancer Centers to initiate research programs to focus on the role of environment in breast cancer. All three institutes are constituents of the National Institutes of Health. This funding allows faculty of the medical schools associated with the Cancer Centers to hold meetings and retreats to develop plans for major research programs on the relationship between environmental agents and breast cancer.

New faculty will be recruited and pilot studies will begin to gather the important data necessary to successfully compete for research funding. The centers are funded so that they may use a multidisciplinary approach in exploring the etiology of breast cancer, drawing on the expertise of basic scientists, clinicians, and epidemiologists. The awards went to Karen Antman of Columbia University, Nancy Davidson of Johns Hopkins University, Ronald Herberman of the University of Pittsburgh, and J. Dirk Inglehart of Duke University.

Center Director Named AAAS Fellow

Daniel W. Nebert, Director of the Center for Environmental Genetics at University of Cincinnati Medical Center, and Director of the NIEHS Environmental Health Sciences Center in Cincinnati, has been elected a fellow of the American Association for the Advancement of Science by the AAAS Council. Each year, beginning in 1874, the council elects members whose contributions to science are distinguished. Nebert was cited particularly for "many contributions to the fields of pharmacogenetics, the cytochrome P450 gene nomenclature system, evolution of drug metabolizing enzymes, carcinogenesis, and environmental toxicology." Nebert's election to this distinguished fellowship was acknowledged by presentation of a certificate and rosette at the AAAS Fellows Forum, February 18, a part of the association's 1995 annual meeting in Atlanta, Georgia.